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Cultural Characteristics for the Enhanced Mycelial Growth of *Ramaria botrytis*

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The culture conditions for the enhanced mycelial growth of *Ramaria botrytis* was investigated. The optimal temperature and pH for the mycelial growth were 24°C and 5.0, respectively. It was shown that starch was best of several carbon sources in Czapek-Dox medium as a minimal medium for the enhanced mycelial growth. Organic nitrogen sources were better than inorganic ones for mycelial growth. The appropriate vitamin and mineral salt were biotin and FeCl3, respectively. When this strain was cultured with $FeCl_3$ for 30 days, 19.23 g/*l* of dry mycelium of *R. botrytis* was obtained.

KEYWORDS: Cultivation, Media, Mycelia, *Ramaria botrytis*

Ramaria botrytis (Pers.: Fr.) Ricken is a kind of ectomycorrhizal fungus growing in autumn in the broadleaved forest, which is widely distributed around the plateaus and mountains of the Eastern Asia, Europe, and North America (Lee, 1988; Park and Lee, 1997). It is distinguished by its size and white to creamy, vinaceous tipped, branches which fade in age. Especially, the vinaceous-tipped coral fruit body has been regarded as an excellent edible mushroom with fruit scent and chicken breast meat taste.

There has, so far, been a few information of the Genus *Ramaria*, e.g., tasty constituents and minerals of *R. botrytis* (Seoh *et al*., 1974; Pyo and Ro, 1975), anticancer activity of *R. formosa* against sarcoma 180 implanted in mice (Yoo *et al*., 1982), immuno-modulating anticancer activity of *R. botyritis* extract (Kim and Jung, 1995; Kim *et al*., 1999) and enzyme activities of the fruit body of *R. botrytis* (Lee and Han, 2001).

However, no information has been available upon the culture condition and physiological characteristics of *R. botrytis*. In this study, cultural characteristics of *R. botrytis* were investigated for artificial cultivation, and food and medicinal use of the mycelia.

Materials and Methods

Strain. The mycelia used in this study were isolated from the fruit body, identified and named as *R. botrytis* DGUM 29001 (Lee and Han, 2001). Recently, the sequences of ITS1, 5.8S and ITS2 of rRNA of *R. botrytis* DGUM 29001 (Accession No. AY588247) was deposited in the GenBank.

Inoculation and cultivation. The mycelia were grown on DTM agar (composition; 20 g of glucose, 30 g of starch, 4.0 g of yeast extract, 2.0 g of soytone, 0.6 g of $MgSO_4$, 0.2 mg of $FeCl_3$, 1 mg of pyridoxine, 15 g of agar and 1.0 *l* of distilled water, pH 5.0) and collected with a cork borer (diam, 5 mm). The mycelia were inoculated into 100 *ml* of DTM broth in 250-*ml* flask and cultivated at 24°C in shaking incubator (120 rpm) for 30 days. For the use of mycelium as an inoculum, the cultured mycelia were homogenized with an electric mixer (Braun Co., model MR-500-MCA).

Determination of mycelial growth. After 30 day-cultivation, the culture broth was filtrated with a filter paper (Toyo No. 2) and washed 3 times with distilled water. Then, the mycelia were dried at 105°C for 24 hr. Dry weight of the cultured mycelia was determined by subtracting the dry weight of a filter paper from the total dry weight (Lee *et al*., 1997).

Screening of favorable optimal media. Seven different culture media were used to investigate a mycelial growth of *R. botrytis* (Table 1). The pH of medium was adjusted to 5.2 with 1.0 N HCl and incubated for 30 days at 24°C.

Measurement of optimal temperature and pH. To determine the optimal temperature for mycelial growth of *R. botrytis*, the cultures in DTM broth were incubated for 30 days at 20, 24, 27. 30 and 34°C, respectively. To determine the optimal pH, DTM broth was adjusted to the range of pH 3.0~9.0 with 1.0 N HCl or NaOH, and incubated for 30 days at 24°C.

Screening of favorable nutrient for culture. To determine favorable nutrient for stimulating the mycelial growth, Czapek-Dox medium (CDM) broth was used as a minimal medium. The malt extract-glucose medium (MEGM) broth was used to determine the effect of vitamin sources

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and mineral salts. Sucrose as a carbon source in the CDM broth was replaced with various carbon sources such as monosaccharose, disaccharide, and polysaccharide. To screen nitrogen sources for enhanced mycelial growth of *R. botrytis*, 12 different nitrogen sources was supplemented to CDM. Carbon and organic nitrogen sources were added at the final concentration of 1.0% and 0.3% (w/v), respectively. Twenty mM of inorganic nitrogen source was used. Various vitamin sources were filtrated with a membrane filer (pore size, 0.2 μm) and then added at the concentration of 0.5 mg/*l*. Mineral salts were added at the final concentration of 0.2 mg/*l*.

Results and Discussion

Determination of complex media. When the mycelia were cultivated at 24°C for 30 days, an excellent mycelial growth was shown in DTM and MEM (Table 1). The YMM, MCM, ACM and GPM media were comparatively effective. However, very little mycelial growth was shown in CDM, which consists of simple carbon, inorganic nitrogen and minerals.

Optimal temperature and pH on the mycelial growth. When the mycelia were cultured at 5 different temperatures for 30 days in DTM broth (pH 5.0), the most effective temperature for the enhanced mycelial growth was 24°C (Fig. 1). However, the mycelial growth was rapidly decreased at above 27°C. This corresponds to the result that the mycelial growth of *Tricholoma matsutake*, an ectomyccorrhizal fungus, also was decreased at above 27°C (Ohta, 1983). When the mycelia were cultured at 24°C for 30 days in DTM broth, the optimal pH for the mycelial growth was 5.0, whereas there was no favorable for mycelial growth at pH 8.0 and pH 9.0 (Fig. 2).

Effect of carbon sources: The CDM was used to determine the effect of carbon source on mycelial growth. As shown in Table 2, starch as a polysaccharide was the best for mycelial growth and dry mycelial weight was 1.23 g/*l*. Also, trehalose as a disaccharide was comparatively effective. Among monosaccharoses tested, glucose and fructose were very good carbon sources. With the carbon source of higher mycelial production, the pellect was smaller in size and compacter in shape. This result was similar to the case of *T. matsutake* reported by Lee *et al*. (1997).

Table 1. Effect of various medium on the mycelial growth of *R. botrytis*

Culture media[a]	Dry mycelial weight (g/*l*)[b]
ACM	3.72±0.72
CDM	1.15±0.18
DTM	7.54±0.34
GPM	2.69±0.22
MCM	4.93±0.33
MEM	6.96±0.22
YMM	5.37±0.26

[a]ACM; *Agrocybe cylindracea* medium, CDM; Czapeck-Dox medium, DTM; Dongguk *Tricholoma matsutake* medium, GPM; glucose peptone medium, MCM; mushroom complex medium, MEM; malt extract medium, YMM; yeast extract-malt extract medium.
[b]Mean±standard deviation for 3 replicates.

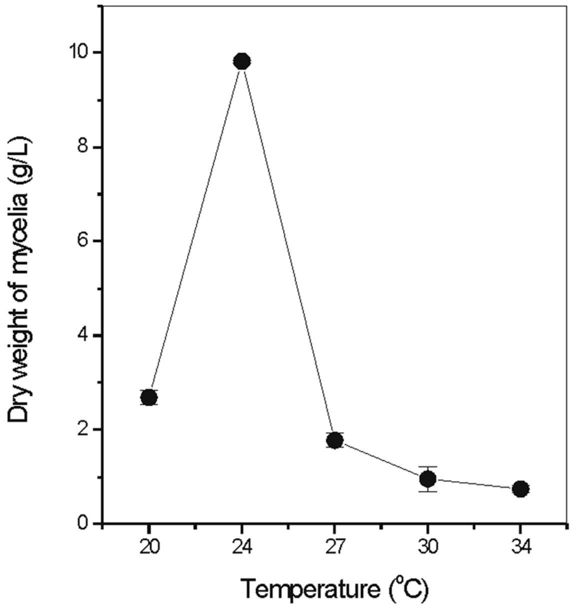

Fig. 1. Effect of temperature on the mycelial growth of *R. botrytis*. The mycelia were cultivated for 30 days in DTM broth (pH 5.0).

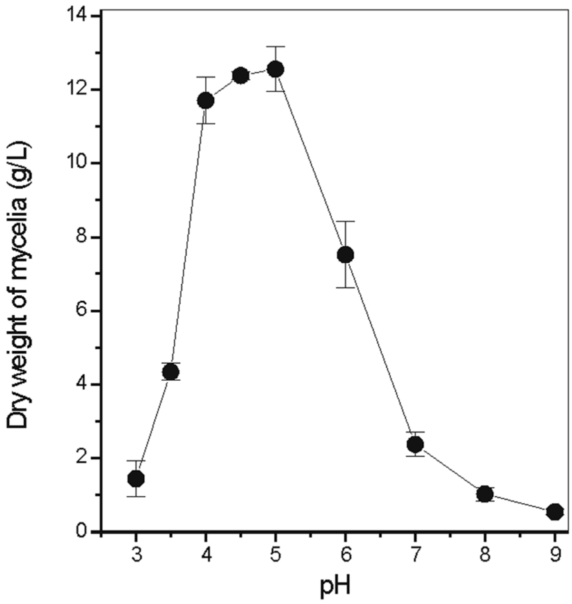

Fig. 2. Effect of pH on the mycelial growth of *R. botrytis*. The mycelia were cultivated at 24°C for 30 days in DTM broth.

Table 2. Effect of carbon source on the mycelial growth of *R. botrytis*

Carbon source[a]	Dry mycelial weight (g/*l*)[b]	Carbon source[a]	Dry mycelial weight (g/*l*)[b]
Fructose	0.93±0.12	Lactose	0.77±0.06
Galactose	0.80±0.04	Trehalose	0.93±0.14
Glucose	1.17±0.12	Maltose	0.85±0.06
Xylose	0.83±0.01	Raffinose	0.92±0.18
Arabinose	0.88±0.06	Starch	1.23±0.03
Glycerol	0.56±0.08	CMC[c]	0.53±0.17
Mannitol	0.27±0.12	Cellulose	0.64±0.04
Sucrose	0.70±0.03	Control[d]	0.85±0.02

[a]The cultivation was carried out at 24°C for 30 days in CDM broth (pH 5.0) supplemented with 1.0% (w/v) each carbon source.
[b]Mean±standard deviation for 3 replicates.
[c]Carboxymethyl cellulose.
[d]Control was tested with CD medium except for carbon source.

Table 3. Effect of nitrogen source on the mycelial growth of *R. botrytis*

Inorganic nitrogen source[a]	Dry mycelial weight (g/*l*)[b]	Organic nitrogen source[a]	Dry mycelial weight (g/*l*)[b]
$(NH_4)_2SO_4$	1.46±0.05	Proteose peptone	1.96±0.28
NH_4Cl	1.21±0.11	Bacto peptone	3.64±0.06
$Ca(NO_3)_2$	1.25±0.29	Yeast extract	3.94±0.18
$NaNO_2$	1.42±0.15	Tryptone	2.37±0.42
$NaNO_3$	0.11±0.11	Malt extract	3.18±0.06
Urea	0.29±0.04	Soytone	5.10±0.45
Control[c]	1.06±0.02	Controlc	1.12±0.19

[a]The cultivation was carried out at 24°C for 30 days in CDM broth (pH 5.0). The final concentration of inorganic and organic nitrogen source were 20 mM and 0.3% (w/v), respectively.
[b]Mean±standard deviation for 3 replicates.
[c]Control was tested with CD medium except for nitrogen source.

Effect of nitrogen sources: In order to determine the optimal inorganic nitrogen source for enhanced mycelial growth of *R. botrytis*, various sorts of inorganic nitrogen were supplemented to CDM. Ammonium sulfate and sodium nitrite were very effective sources of inorganic nitrogen. However, urea and sodium nitrate did not enhance the mycelial growth (Table 3). When various organic nitrogen sources were added to CDM, yeast extract, bacto-peptone and malt extract were most appropriate ones. Tryptone and proteose peptone were effective. When *R. botrytis* was cultured with 0.3% of soytone for 30 days, 5.10 g of dry mycelium was produced per liter (Table 3).

Effect of vitamin and mineral salts: When various vitamins were added to the DTM, biotin and folic acid were very effective on the mycelial growth of *R. botrytis*. However, nicotinic acid and *p*-aminobenzoic acid were not effective (Table 4). $FeCl_3$ was an excellent sources of mineral salts and $FeSO_4$ and $MgSO_4$ were very effective (Table 4). For preparation of optimal medium of artificial cultivation, the sources and concentrations of each nutrition in medium remains still to be studied.

Table 4. Effect of vitamin and mineral salt on the mycelial growth of *R. botrytis*

Vitamin[a]	Dry mycelial weight (g/*l*)[b]	Mineral salt[a]	Dry mycelial weight (g/*l*)[b]
Riboflavin	7.32±0.36	$FeSO_4$	13.72±0.32
Pyridoxine	7.15±0.51	$MgSO_4$	15.91±0.75
PABA[c]	6.98±0.14	$FeCl_3$	19.23±0.72
Thiamine	7.23±0.25	Control[d]	11.18±0.29
Folic acid	7.55±0.39	Nicotinic acid	6.80±0.27
Biotin	7.62±0.33	Control[d]	7.03±0.22

[a]The cultivation was carried out at 24°C for 30 days in MEGM broth (pH 5.0). The final concentration of vitamin and mineral salt were 0.5 mg/*l* and 0.2 mg/*l*, respectively.
[b]Mean±standard deviation for 3 replicates.
[c]PABA; p-amino benzoic acid.
[d]Control medium was composed of peptone 5 g, malt extract 20 g, glucose 20 g and D. W. 1 *l*.

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